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Pathological Highlights from the 2011 Growing Season

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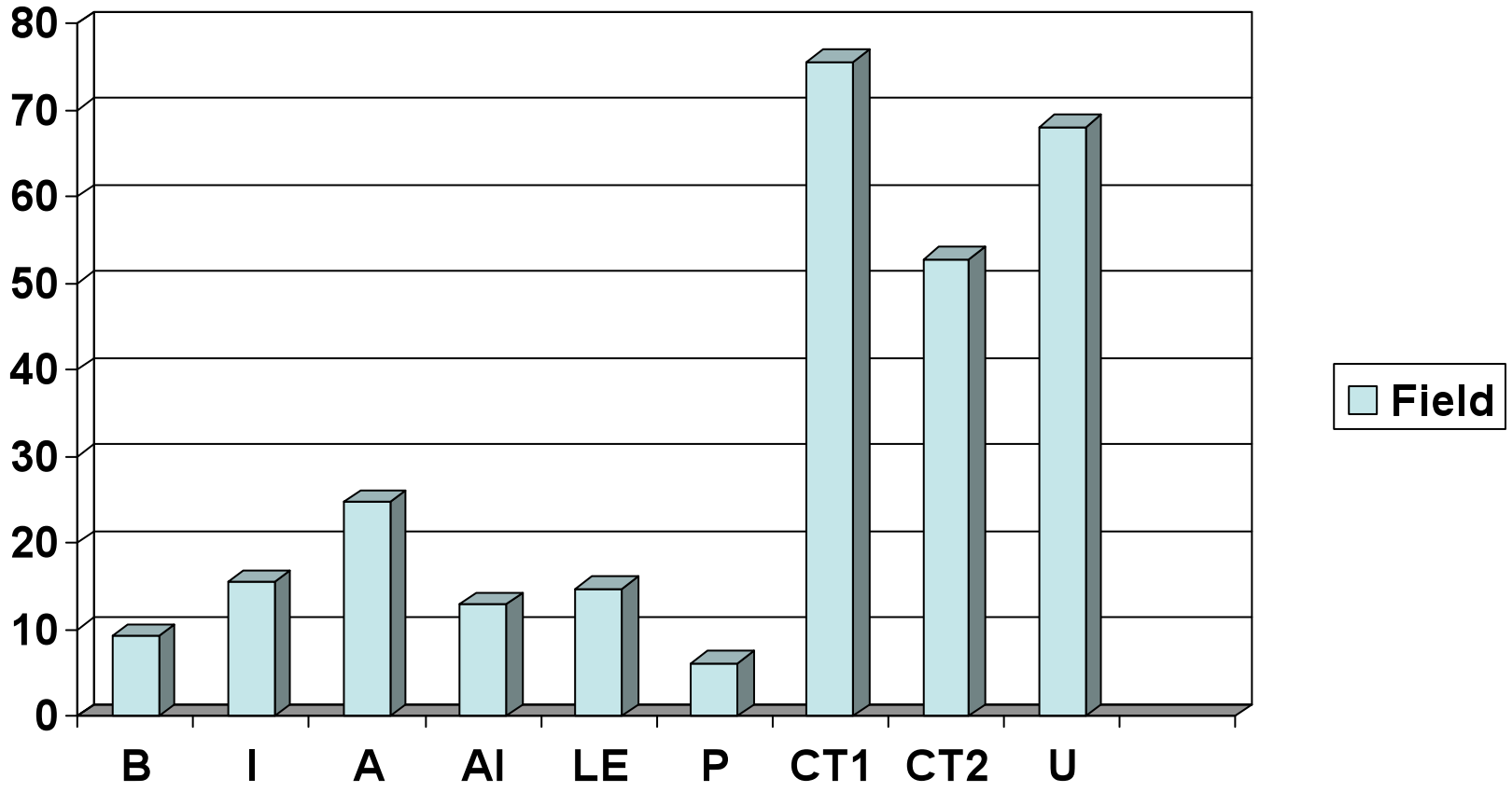
Projects to discuss

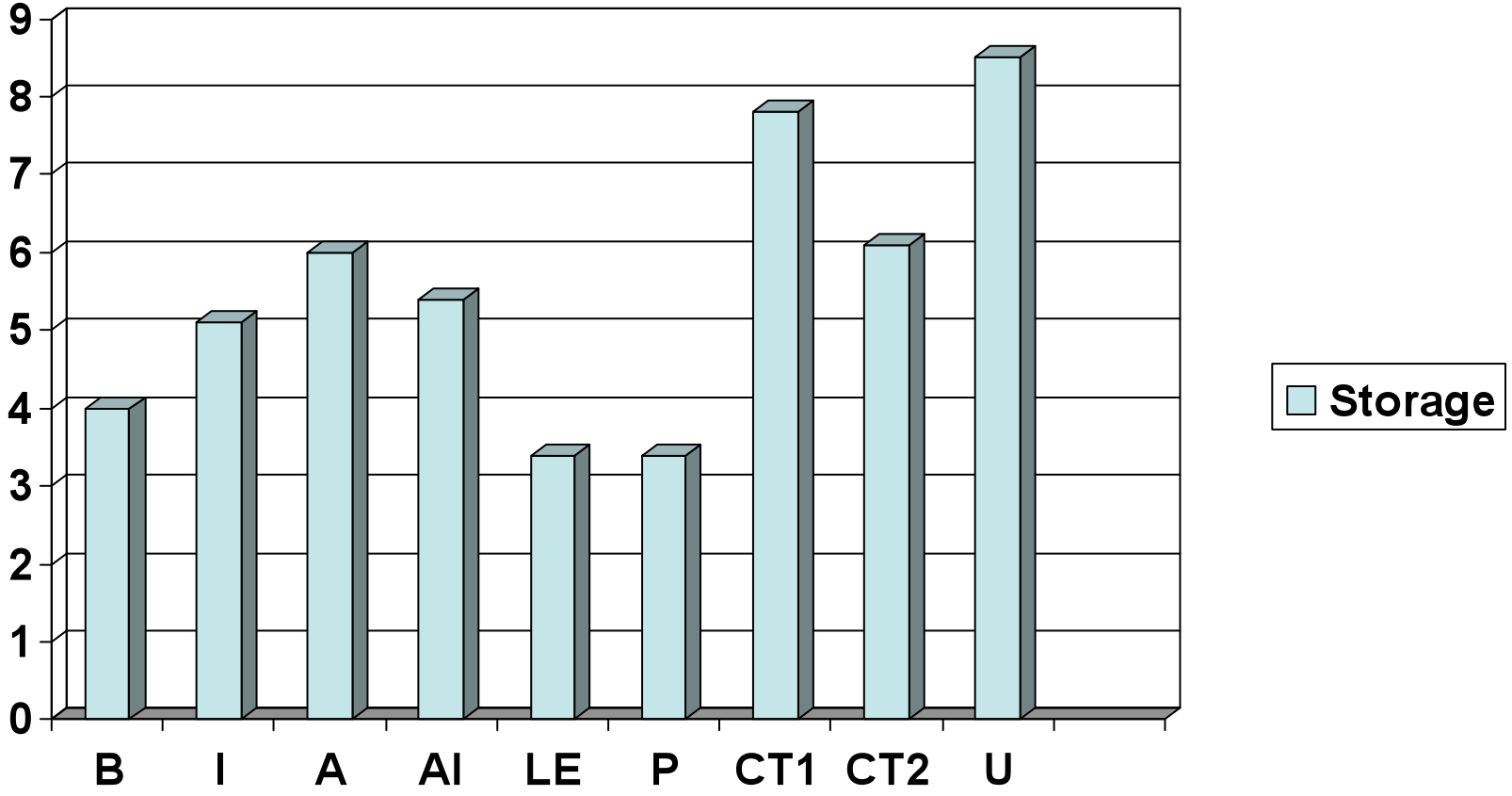
- New formulations fungicide field trial
- Isolations of fungi from different beds experiencing severe leaf spot – second year results
- Upright dieback project summary and inoculations with *Fusicoccum putrefaciens*
- Potential biocontrol agents in wild beds
- Loosestrife disease
- Dodder disease

New formulations fungicide field trial

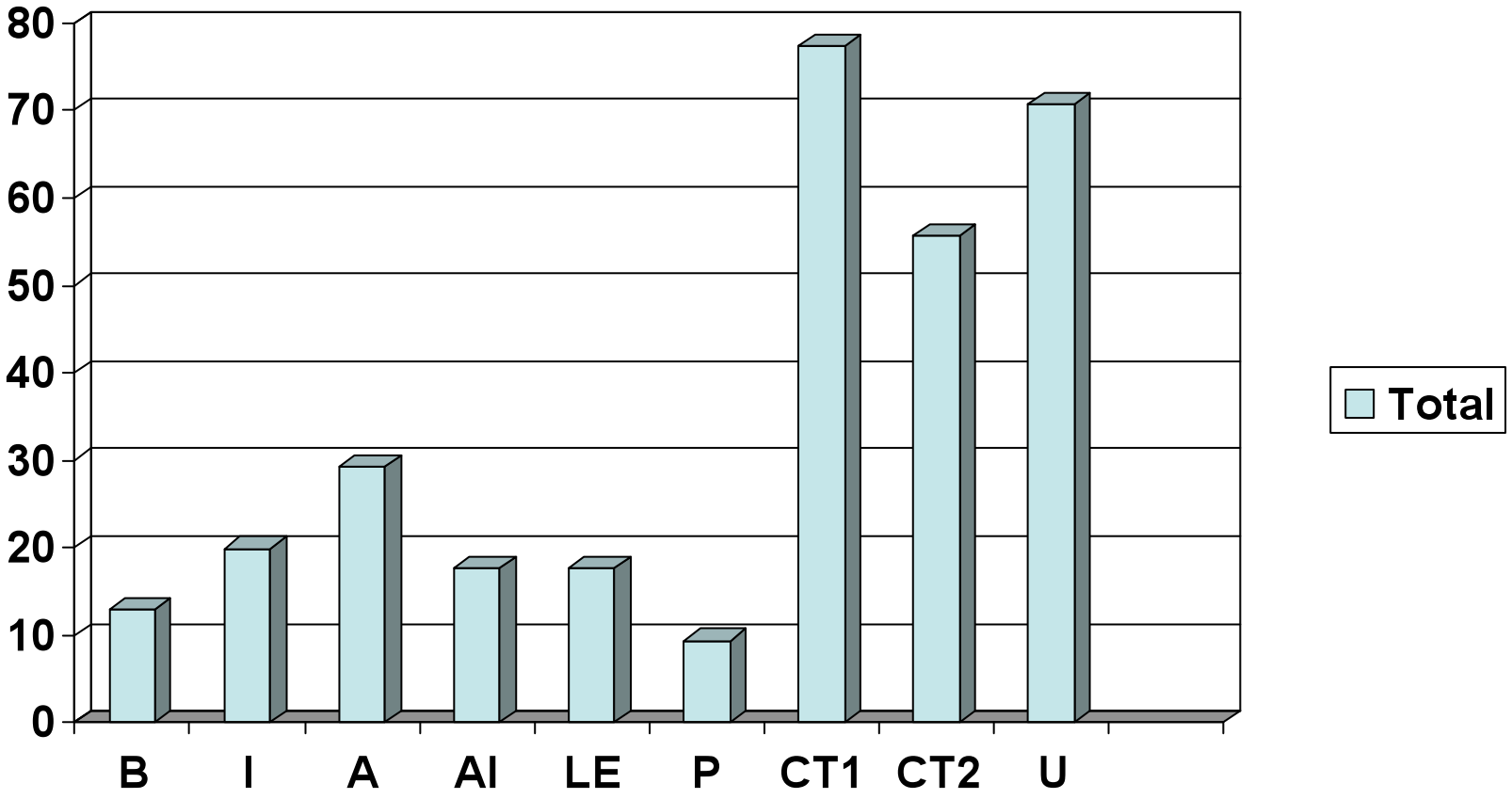
New formulations field rot – 2011



New formulations storage rot – 2011



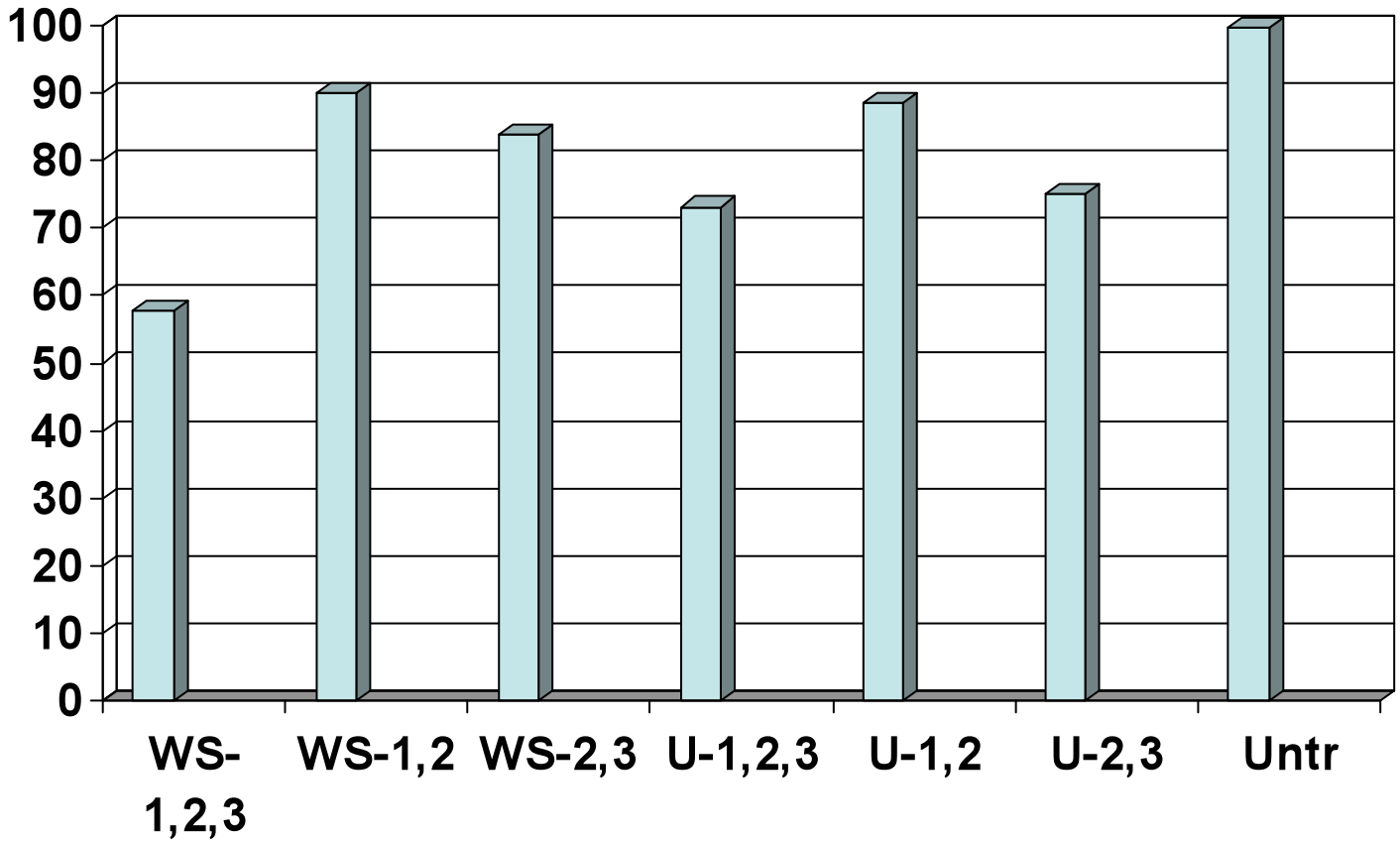
New formulations total rot – 2011



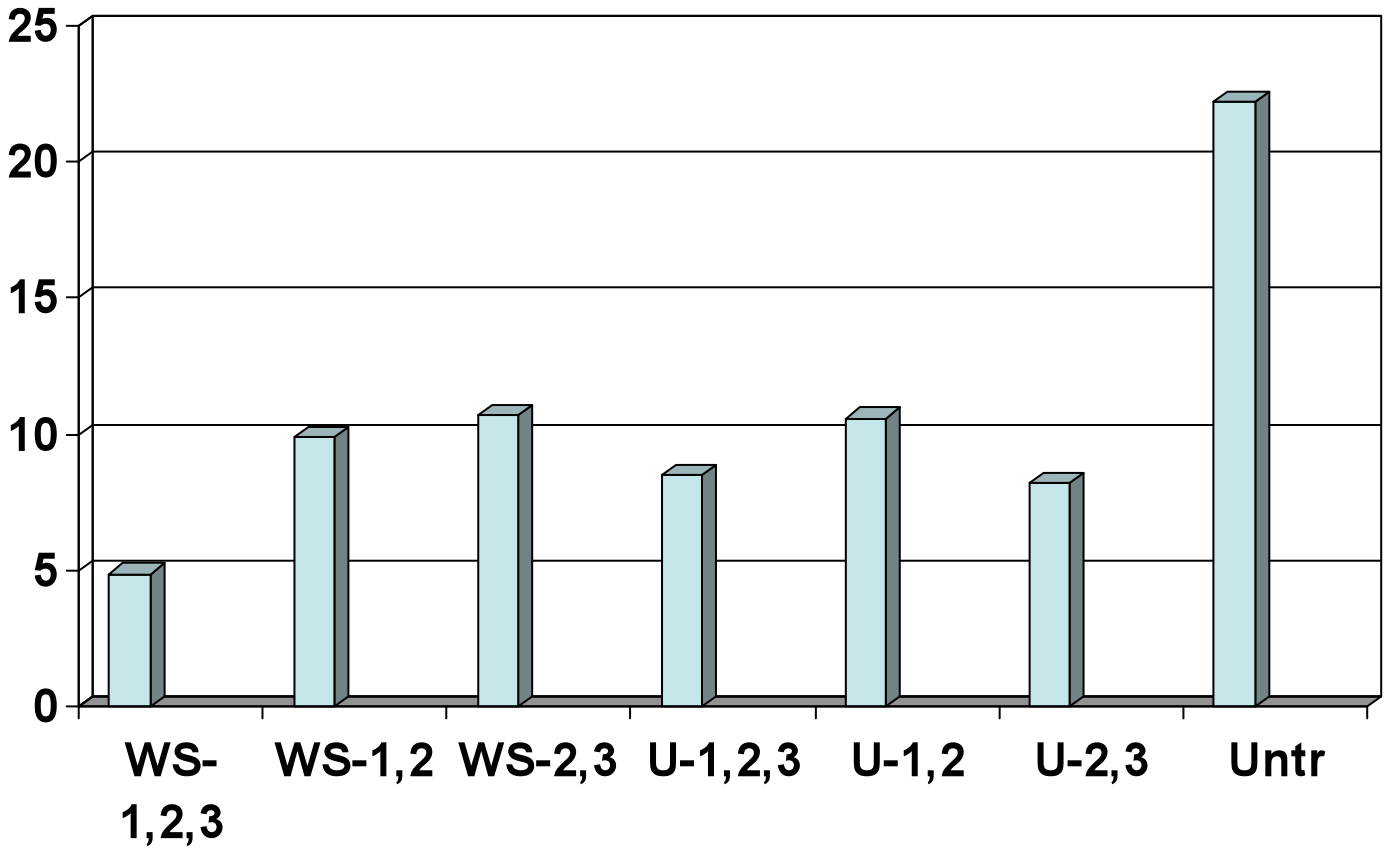

Previous two years received Indar + Abound
Note increased bloom

Sometimes bizarre unexplainable fruit rot situations occur for researchers too

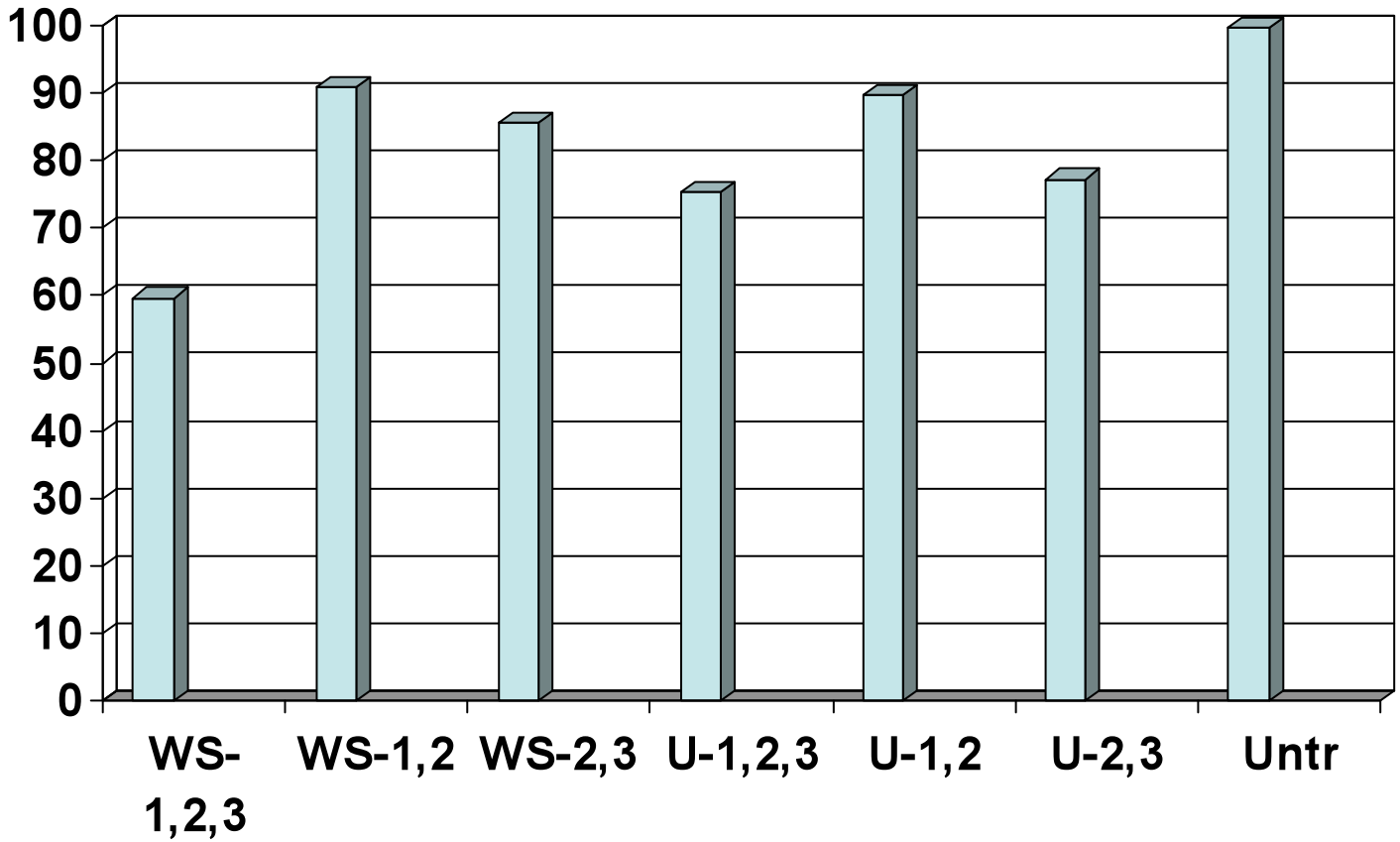
2011 Bravo field trial – Field rot



2011 Bravo field trial – Storage rot



2011 Bravo field trial – Total rot



Isolations of fungi from different beds experiencing severe leaf spot – second year results

Fungi cultured from leaf spots

- *Phyllosticta vaccinii* – 63.3%***
- *Colletotrichum acutatum* – 20.0%**
- *Fusicoccum putrefaciens* – 0%*
- *Pestalotia vaccinii* – 24.7%
- *Physalospora vaccinii* – 5.3%
- *Phomopsis vaccinii* – 7.3%

Fungi cultured from rotted berries

- *Phyllosticta vaccinii* – 38.7%***
- *Colletotrichum acutatum* – 8.0%**
- *Fusicoccum putrefaciens* – 2.7%*
- *Pestalotia vaccinii* – 17.3%
- *Physalospora vaccinii* – 6.0%
- *Phomopsis vaccinii* – 32.0%

Fungi cultured from healthy berries

- *Phyllosticta vaccinii* – 29.0%***
- *Colletotrichum acutatum* – 3.0%**
- *Fusicoccum putrefaciens* – 2.0%*
- *Pestalotia vaccinii* – 16.0%
- *Physalospora vaccinii* – 15.0%
- *Phomopsis vaccinii* – 21.0%


Leafspotting in Demoranville, Quebec


Leafspotting in Demoranville, Quebec

Leafspotting in Demoranville, Quebec

Upright dieback project summary and inoculations with *Fusicoccum putrefaciens*

Ratio of fruiting to vegetative uprights

Bed	08	09	10	11
Bed #1	1/100	1/500	1/1	---
Bed #2	1/50	1/1	1/100	---
Bed #3	1/400	1/5	1/200	1/500

Incidence of *Phomopsis vaccinii*

Bed	08	09	10	11
Bed #1	V – 26 F – 58	V – 16 F – 70	V – 40 F – 62	---
Bed #2	V – 54 F – 50	V – 82 F – 96	V – 50 F – 74	---
Bed #3	V – 38 F – 26	V – 74 F – 94	V – 26 F – 38	V – 26 F – 34

Incidence of *Fusicoccum putrefaciens*

Bed	08	09	10	11
Bed #1	V – 2 F – 2	V – 36 F – 20	V – 10 F – 4	---
Bed #2	V – 2 F – 2	V – 2 F – 4	V – 0 F – 0	---
Bed #3	V – 0 F – 0	V – 10 F – 6	V – 6 F – 16	V – 16 F – 18

Summary of four year findings

- Upright dieback occurs regularly in some beds, is sporadic in other beds
- Early Black is the cultivar most affected by the disease
- The ratio of fruiting to vegetative varies from 1:1 to 1:1000 and varies significantly in a bed from year to year
- In freshly killed uprights, *Phomopsis* occurs at significantly higher levels than *Fusicoccum*
- In overwintered uprights, *Fusicoccum* occurs at higher levels than *Phomopsis*
- *Colletotrichum acutatum* may also be a causal agent of the disease

Pathogenicity tests with *Fusicoccum putrefaciens*

- Three isolates of the fungus cultured from symptomatic uprights from three different beds
- Fungus grown on potato dextrose agar
- Mycelium placed on upright after pin prick, then wrapped in moist cheesecloth under Parafilm
- Controls consisted of agar instead of fungal inoculum

Pathogenicity tests with *Fusicoccum putrefaciens* cont.

- Plants incubated in a growth chamber for 2-3 months with appropriate photoperiod and temperature
- Plants scored for infection
- Tissue pieces selected, surface sterilized and plated on ACMA
- Plates scored at 3 weeks for presence of *Fusicoccum*


R4

Inoculations with *Fusicoccum* Uprights with symptoms Trial #1 and #2

- Control = 4/15 dead
- 009-2 = 11/15 dead
- 009-3 = 8/15 dead
- 010-1 = 9/15 dead

- Control = 6/15 dead
- 009-2 = 15/15 dead
- 009-3 = 15/15 dead
- 010-1 = 8/15 dead

Inoculations with *Fusicoccum Isolations* from uprights Trial #1 and #2

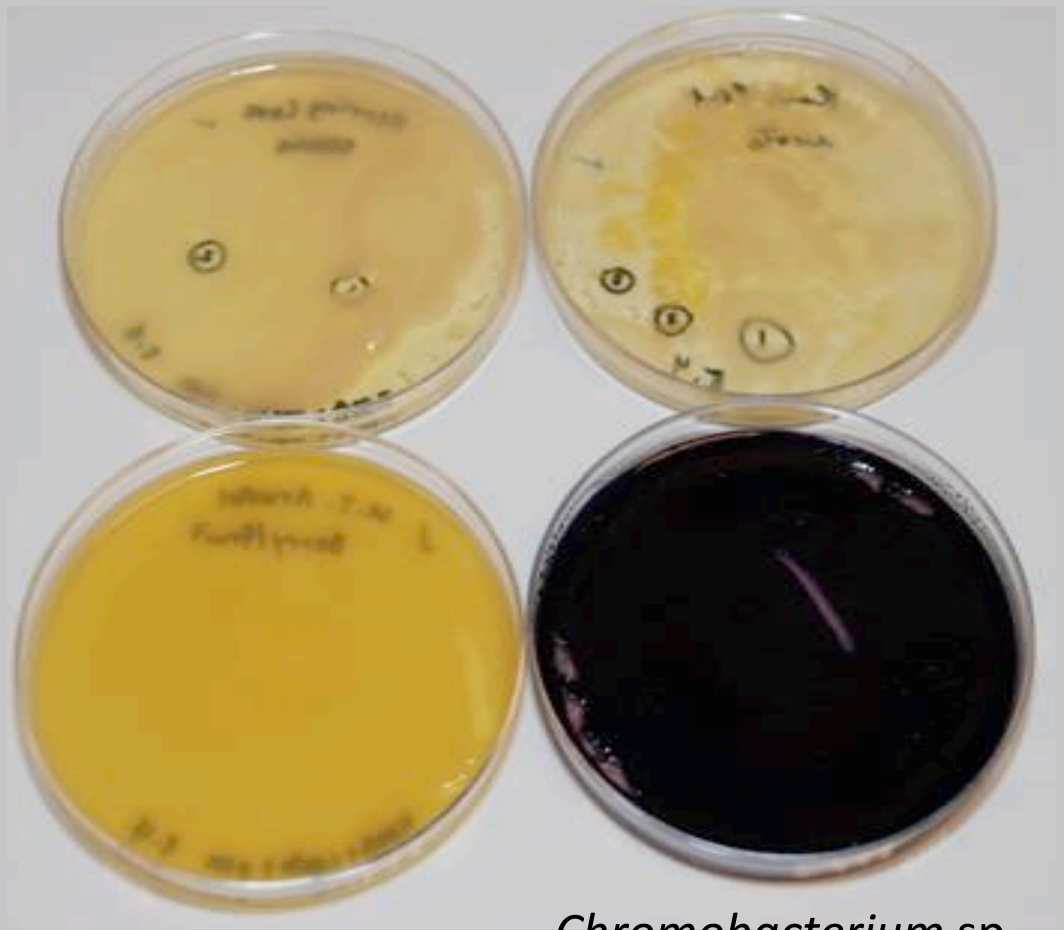
- Control = 0%
- 009-2 = 8.0%
- 009-3 = 10.5%
- 010-1 = 8.7%

- Control = 0%
- 009-2 = 66.7%
- 009-3 = 71.4%
- 010-1 = 4.5%

Potential biocontrol agents in wild beds

Bacteria isolated from native cranberry roots and soil

A large number of bacterial types were isolated from native cranberry roots and associated soil. *Chromobacterium* sp., a dark-purple pigmented bacterium was isolated from all native and commercial cranberry roots and soils, and from the irrigation pond for State Bog.

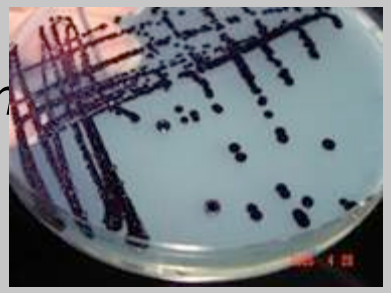


Chromobacterium sp.

Types of bacteria isolated from cranberry plants and soils with potential for biocontrol —so far

- Fluorescent *Pseudomonas*
  - Plant growth-promoting production of IAA
  - Antifungal activity *in vitro*
  - No ice nucleation activity

- *Chromobacterium vaccinium*
  - Inhibits growth of *Phytophthora* and *Thanatophytum*
  - Nematicidal (*C. elegans*)
  - Being tested by USDA for insecticidal activity



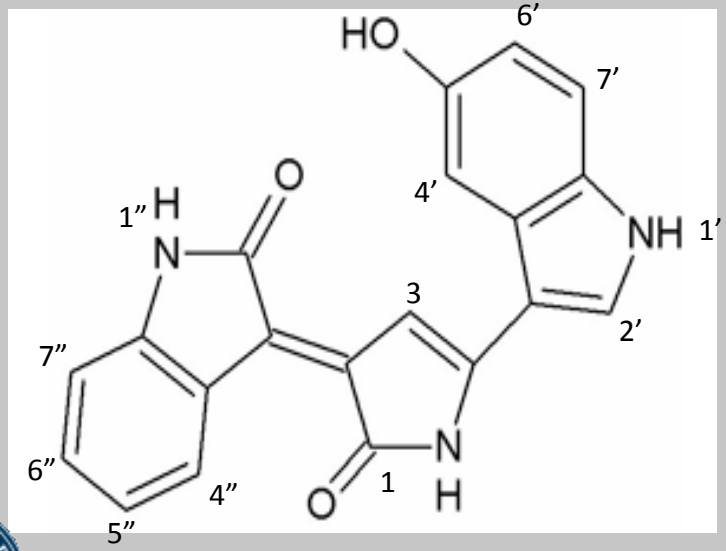
- *Serratia proteamaculans*
  - Known parasite of coleopteran grubs feeding on wheat roots
  - Being tested by USDA for insecticidal activity

Taxonomic structure of cranberry *Pseudomonas* isolates

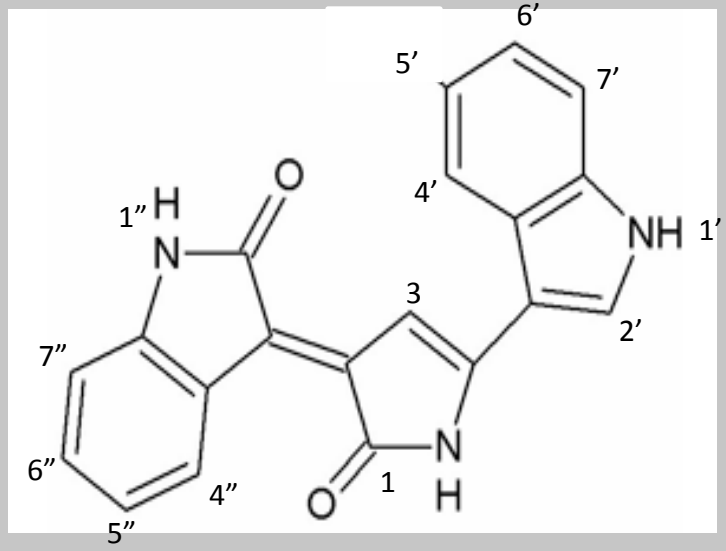
- Every one of the *Pseudomonas* isolates has been genetically tested.
- None of the isolates belongs to a recognized ‘species’ of *Pseudomonas.*
- The *Pseudomonas* bacteria growing on and around cranberries may thus be a unique collection of previously unknown species.
- One isolate (MWU 341) synthesizes large amounts of IAA
  - Auxins are involved in plant development, and are particularly important for fruit development and root growth

Chromobacterium and violacein

- Violacein and have antimicrobial, anti-fungal and anti-cancer activity
- Both are produced by cranberry *Chromobacterium* (*sensu lato*) isolates

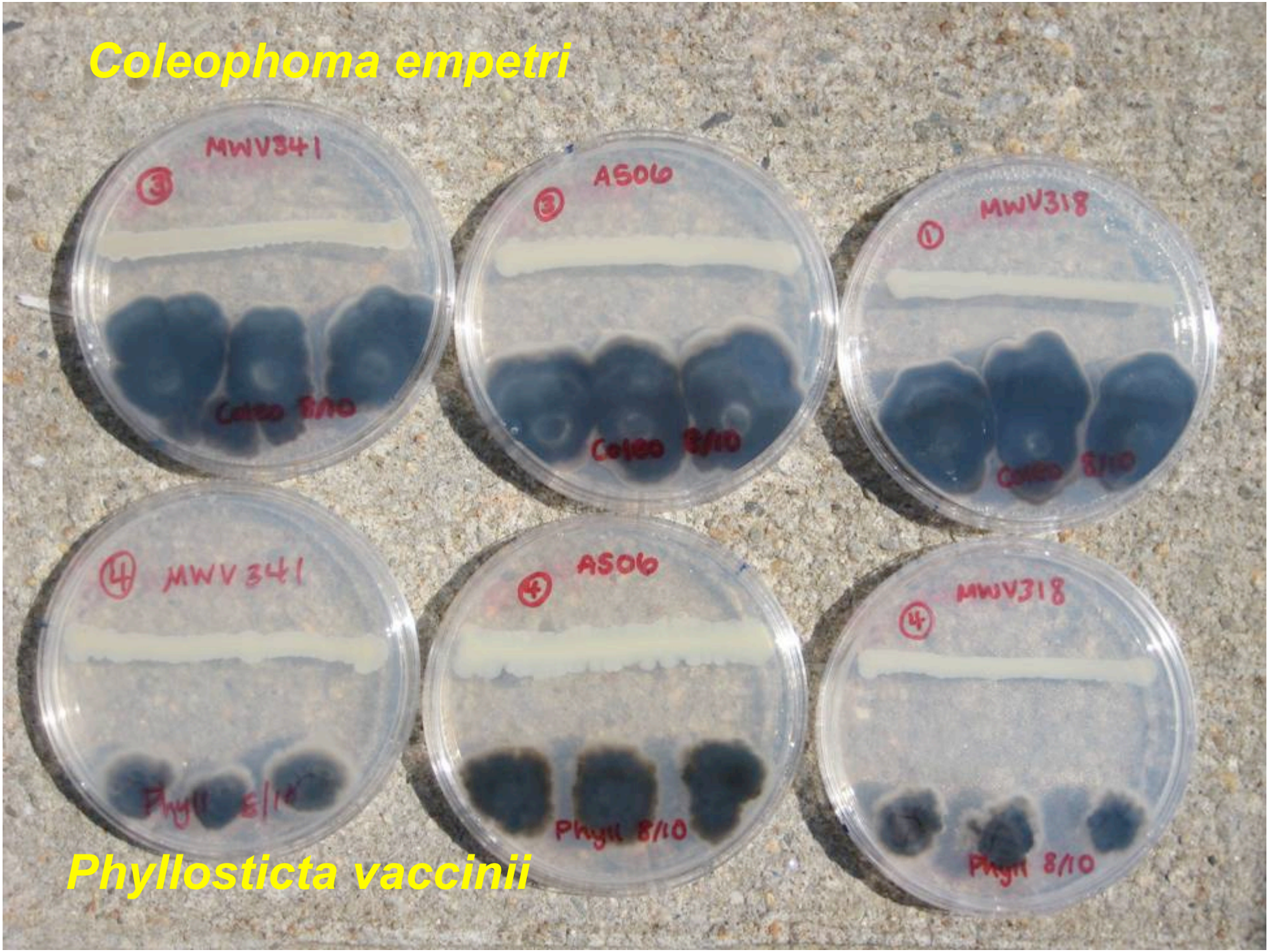


Violacein



Deoxyviolacein

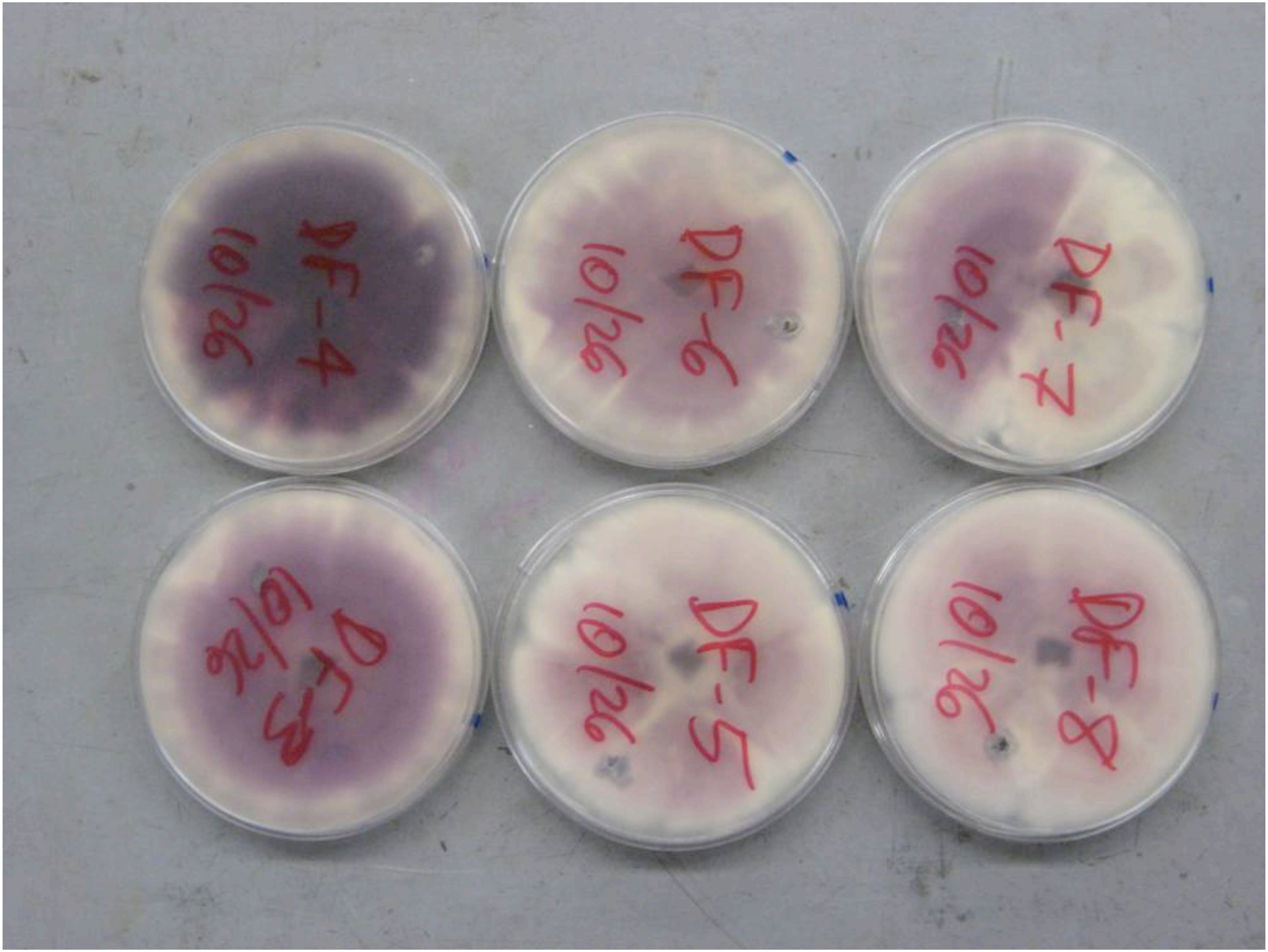

Coleophoma empetri
MWV341
A506
MWV318
MWV341

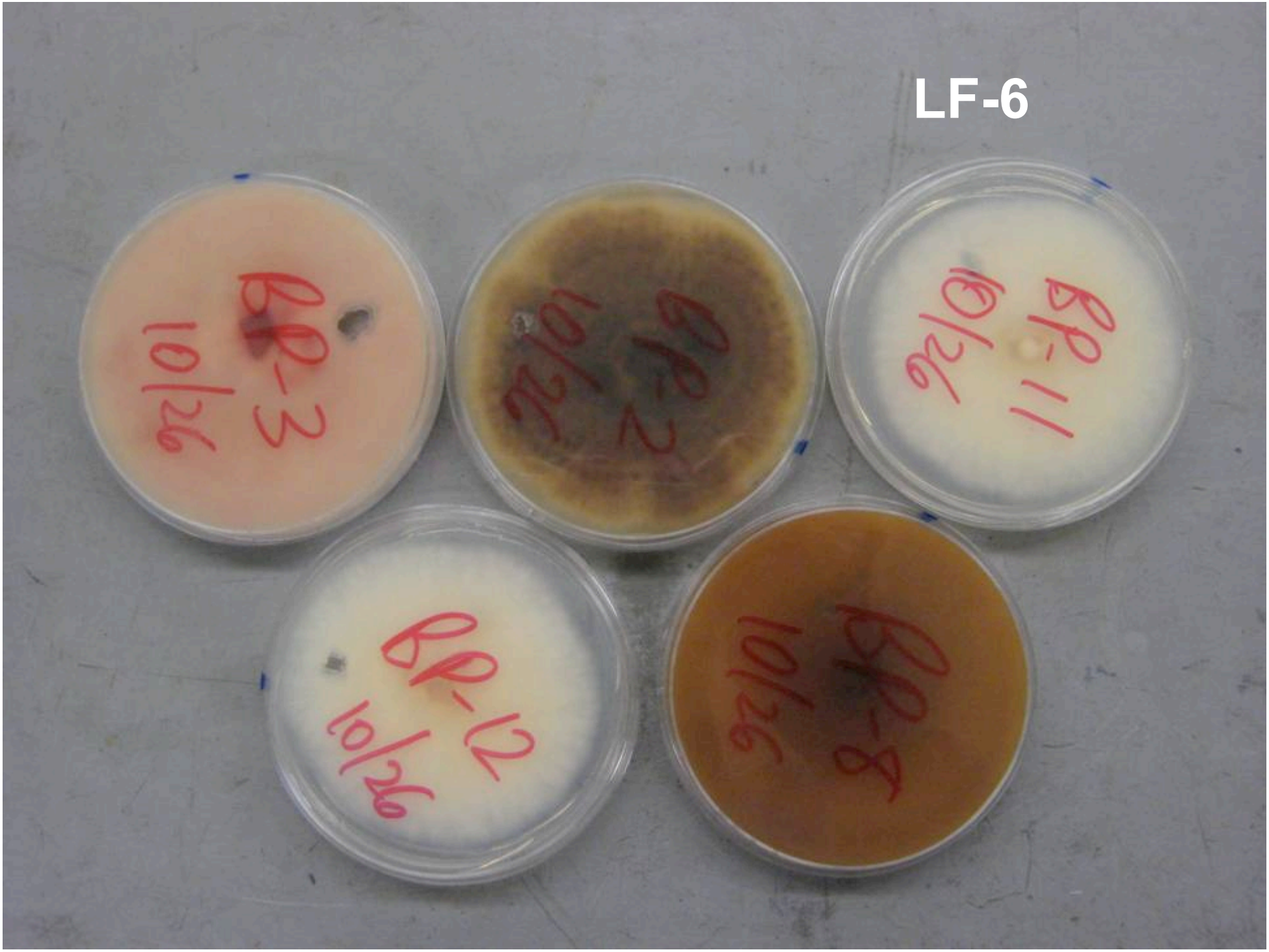

Coleophoma empetri
MWV341
AS06
MWV318
MWV341
AS06
MWV318
Phyll 8/10
Phyllosticta vaccinii

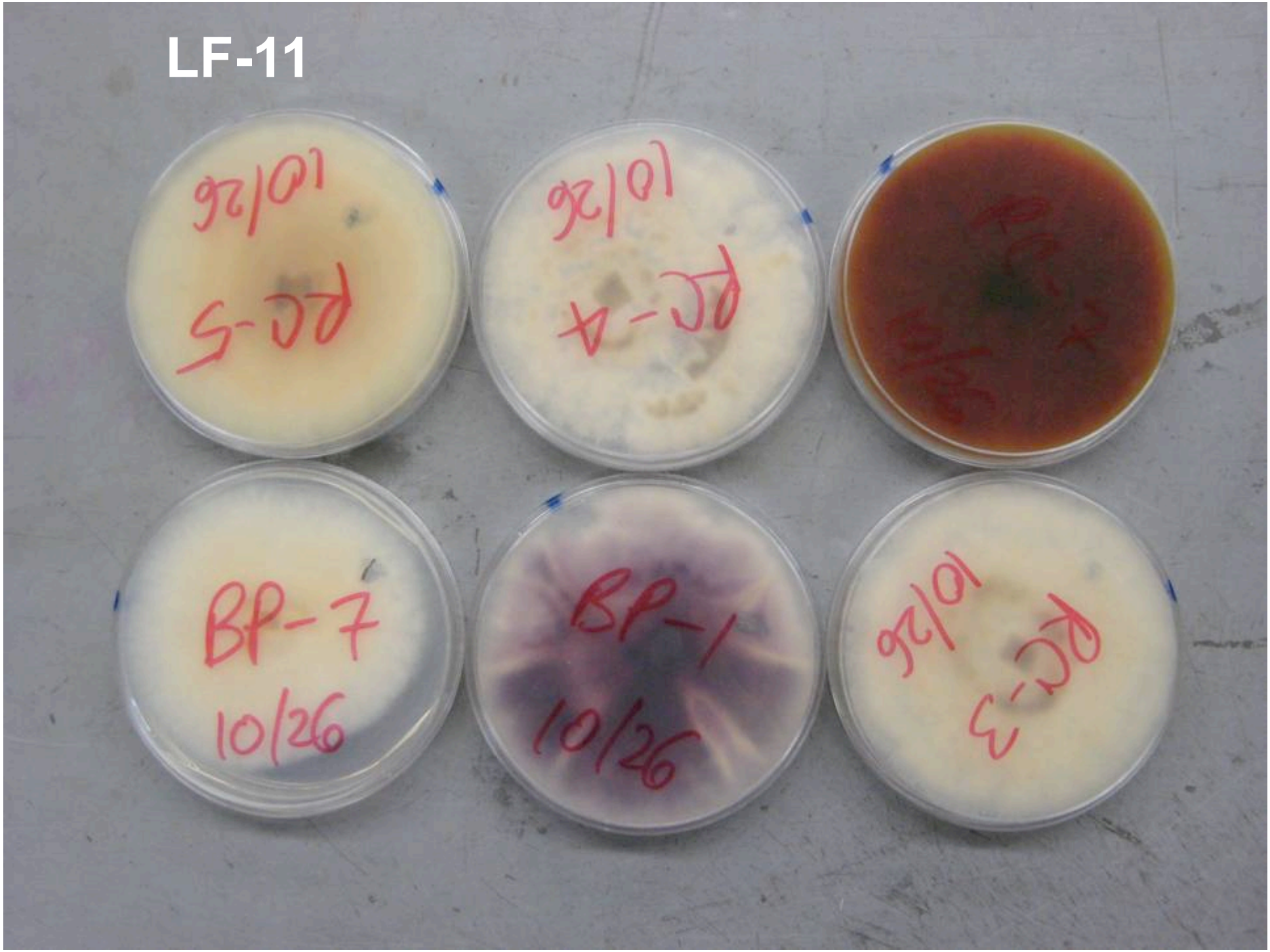
Next steps:

- Continue to identify the bacteria to genus and species
- Select best candidates for antagonism assays using fungal pathogens causing fruit rot, root rot, upright dieback and fairy ring
- Select best antagonists for greenhouse studies and subsequent field trials

Loosestrife disease


DF-4
10/26
DF-6
10/26
DF-7
10/26
DF-3
10/26
DF-5
10/26
DF-8
10/26


LF-6
BP-3
10/26
BP-2
10/26
BP-11
10/26
BP-12
10/26
BP-8
10/26


LF-11
RC-5
10/26
RC-4
10/26
BP-7
10/26
BP-1
10/26
RC-3
10/26

Initial observations

- Affected loosestrife found in four beds – North Carver, Marion, Kingston, South Middleboro
- All characteristically had white mycelium on the underground portion of the plant
- 14/34 isolates determined to be *Fusarium oxysporum*; other 20 were other *Fusarium* species (to be determined by Dr. Wade Elmer)

Inoculum preparation

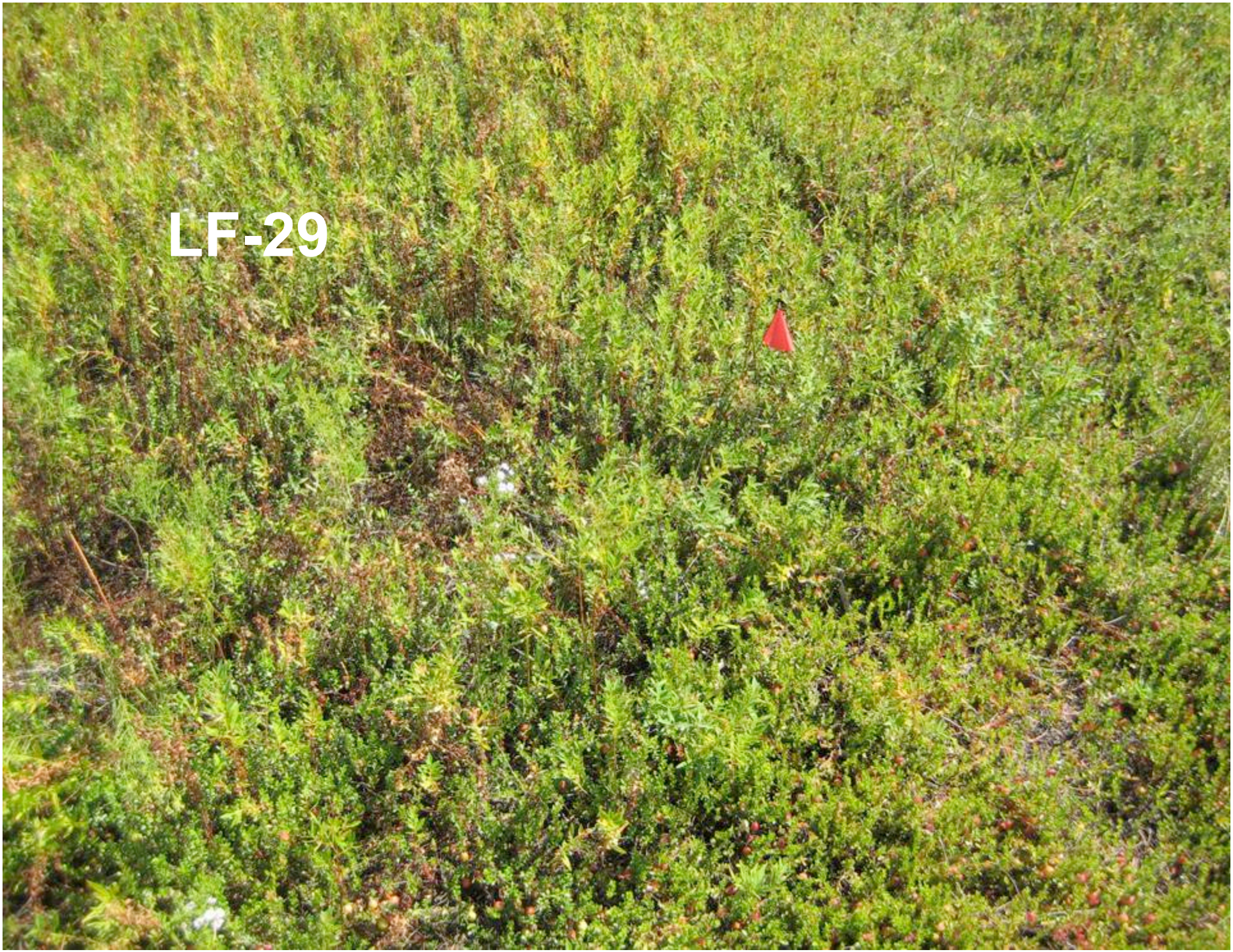
- Mycelium used to inoculate twice autoclaved white millet seed (100 grams seeds in 100 ml water)
- Incubated for 7-10 days, daily shaking the flask to redistribute the fungus
- Duplicate flasks per isolate – one was air-dried, the other was used wet

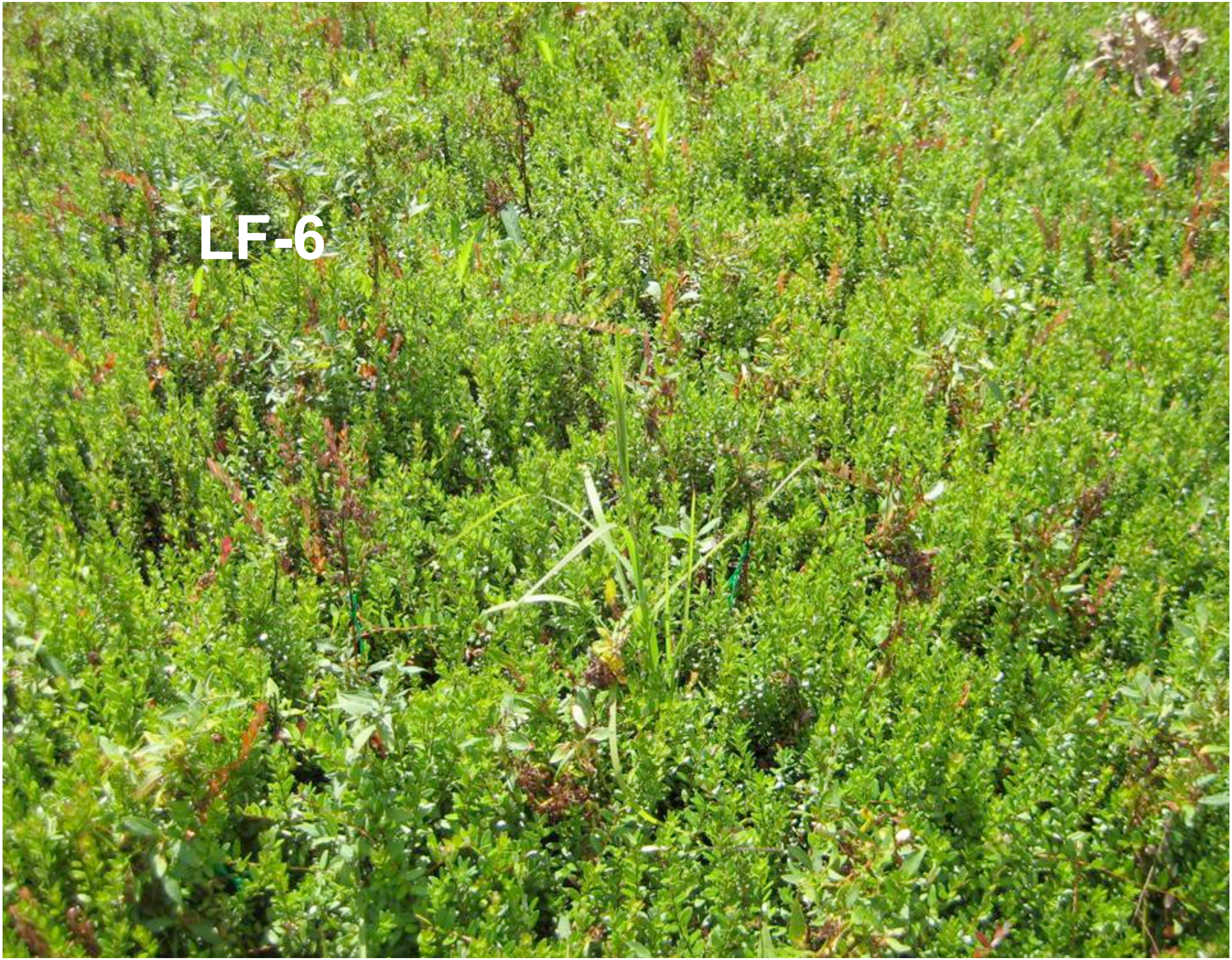

LF-9 W
LF-9 W
LF-32 W
LF-32 W
LF-2 W
LF-29
CONTROL

Field plots

- Four locations – East Freetown and Middleboro (pair 1); South Middleboro and Plymouth (pair 2)
- Nine different isolates used per pair
- Each bed had dry inoculum and wet inoculum in separate areas
- 15-20 tagged plants used per isolate per dry or wet inoculum
- Teaspoon of inoculum inserted at the base of each plant where soil had been removed
- Grower briefly irrigated right before and after
- Only fungicide avoided was Abound


LF-29


LF-29


LF-6


LF-6


LF-11


LF-11

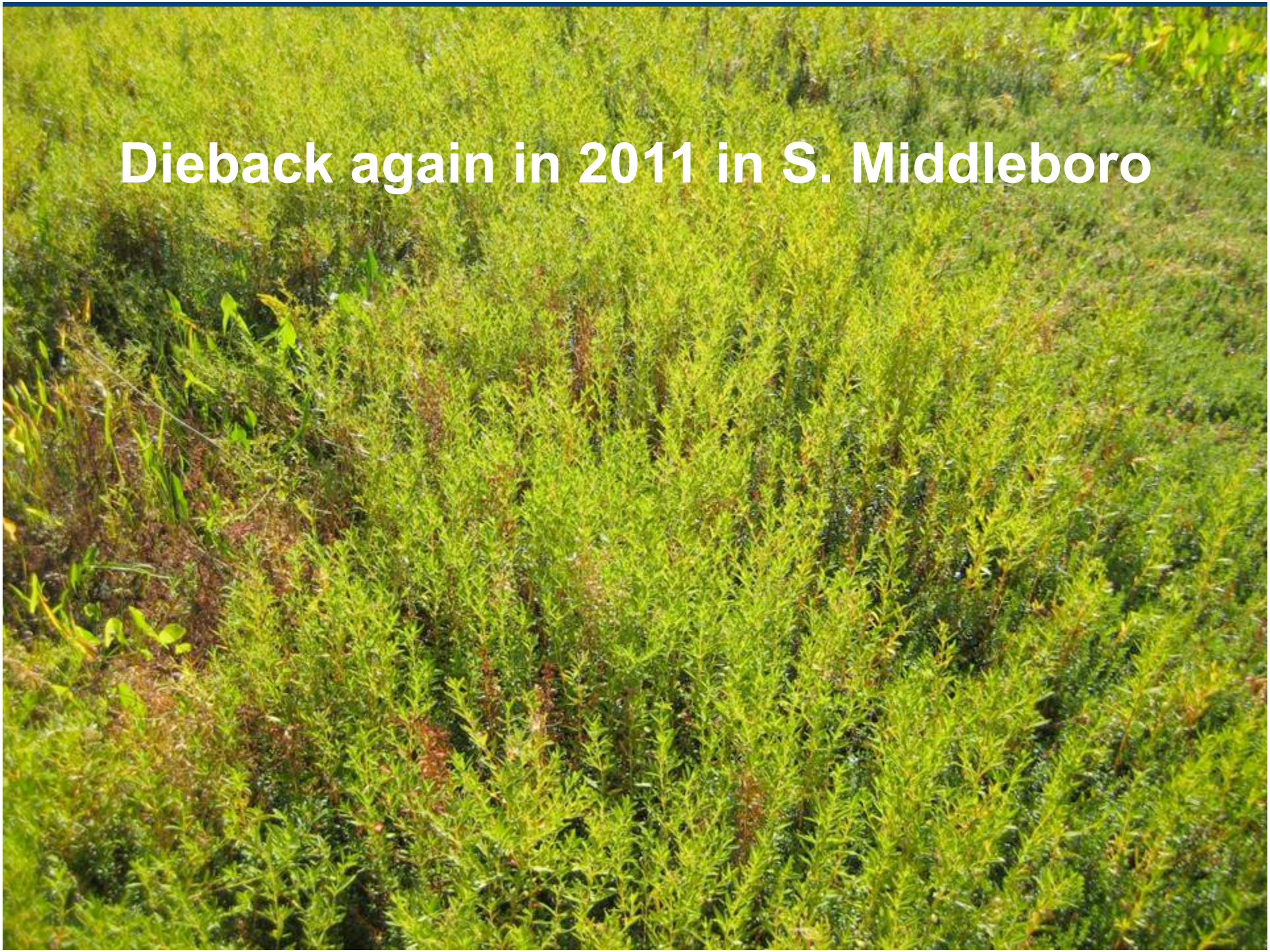

Dieback found in July


No visible white mycelium


More dieback found in July


Injury by borers


Salt injury from TS Irene


Dieback again in 2011 in S. Middleboro


White mycelium again found

Next steps:

- Identify *Fusarium* to species – Dr. Elmer
- Grow up inoculum of selected *Fusarium* isolates, based on field inoculations
- Inoculate loosestrife seedlings in the greenhouse and look for typical symptoms
- Culture same *Fusarium* isolate from infected plants to confirm pathogenicity
- Inoculate loosestrife in the field with selected isolates

Dodder disease


Diseased area

Dodder mycoherbicide trials

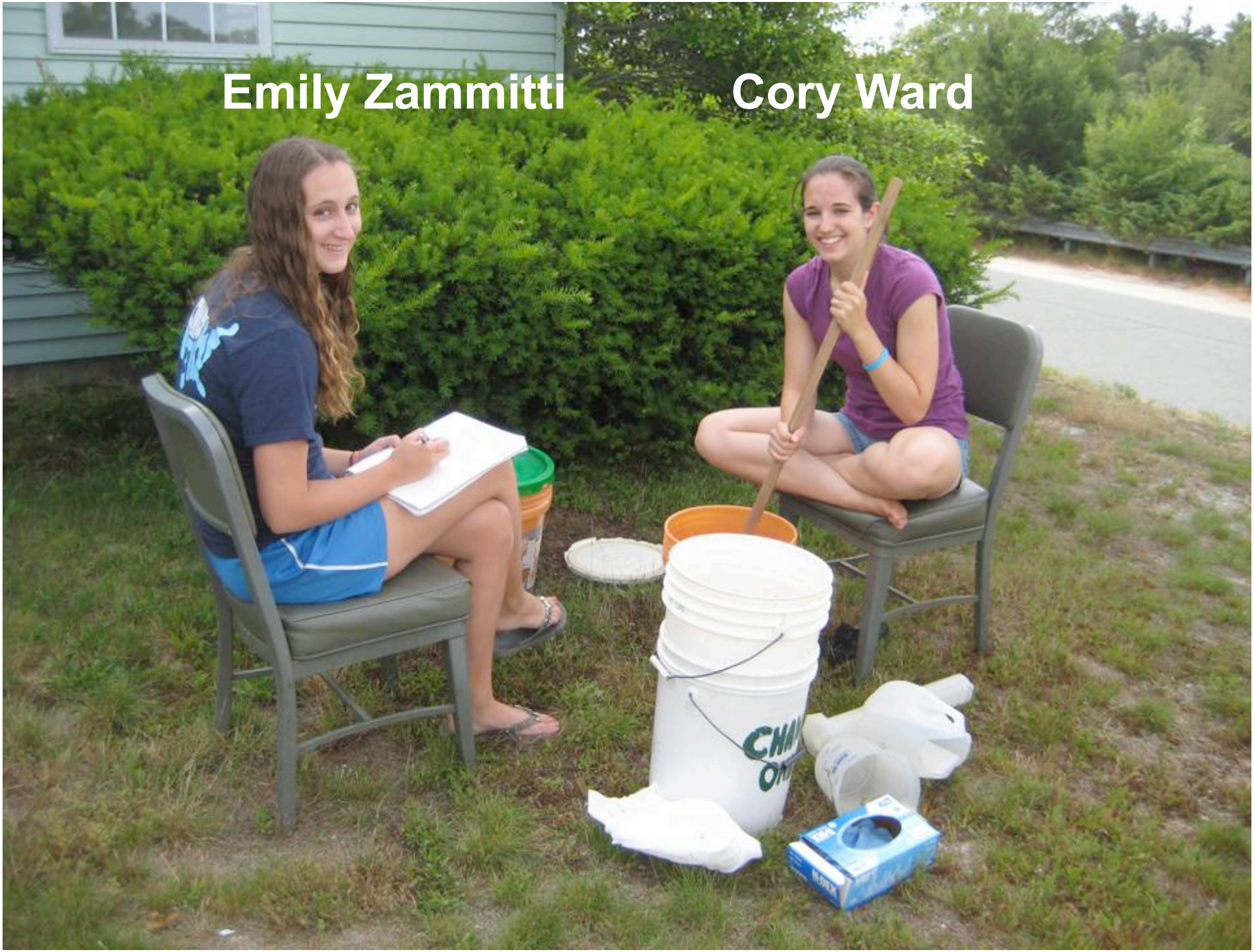
- *Colletotrichum acutatum* (6 isolates) and *Colletotrichum gloeosporioides* (1 isolate)
- Three locations – Wareham, Carver, Centerville
- Dodder inoculated in late June/early July by spraying conidial suspension when relative humidity was high (4 reps/isolate)
- Infection evaluated 2-3 weeks after inoculation

Dodder infection ratings

	Ca1	Ca2	Ca3	Ca4	Ca5	Ca6	Cg1	Con
Bed #1	1.8	2.5	2.5	2.3	1.5	1.3	2.8	1.8
Bed #2	1.8	2.9	0.5	1.9	1.0	2.1	2.0	1.3
Bed #3	2.8	2.9	3.0	2.1	1.5	2.8	3.5	1.6
Avg	2.08	2.75	2.00	2.09	1.33	2.04	2.75	1.54

Thanks!!!

- Lab and field assistance: Lynne Dowdall, Emily Zammitti, Cory Ward and Alex Ward
- Funding: CCCGA; Cranberry Research Foundation; Ocean Spray Cranberries, Inc.; EPA Region 1; USDA/CSREES Special Grant; Dow AgroScience
- Numerous cooperating growers


Emily Zammitti
Cory Ward

Questions?? And GO PATS!!!